Rapid Test Kit for Quantifying Hormonal Activity in Animal Feeding Operation Wastewater

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Problem Statement

•The widespread discovery of pharmaceuticals in wastewater effluents and surface waters across the U.S. is potentially alarming:

- •Estrogenic activities have been found in several effluents which altered vitellogenin induction, hormone titers, gonadal histology, and secondary sexual characteristics in caged fathead minnows.
- •Because of the pharmaceuticals and hormones used in the confined animal feeding operations for poultry, cattle, and swine, the potential release into the environment is high due to highly refractory nature of these materials.
- •Combined with high potencies of the pharmaceuticals and potential positive interactions with other waste materials, these substances have the potential to induce serious impact on resident aquatic life.

•No standardized and well-validated technology is available to screen the effects of contaminants found in animal feeding operation waste on aquatic vertebrate animals residing near the discharge.

•Advanced development and reproduction are controlled in part by the endocrine system which is a primary target of endocrine disrupting chemicals (EDCs).

•The primary goal of the proposed research is to standardize, validate and commercialize an *in vitro* amphibian oocyte maturation model using *Xenopus* as a system for evaluating presently unknown ecological hazards produced by animal feeding operations.

- •The proposed test kit should provide a valuable means of testing for EDC activity and effects.

Technology Description

•The maturation of the amphibian oocyte represents the final stage of oogenesis which ultimately prepares the oocyte for fertilization. Oocyte maturation is marked morphologically by germinal vesicle breakdown (GVBD), and is induced by either progesterone or androgens.

- •Thus, maturation of the oocyte could potentially be disrupted by EDCs. Disruption of oocyte maturation events in *Rana pipiens* and *X. laevis* by estradiol and a synthetic estrogen has been demonstrated previously (Fort et al., 2006).

•A test kit which measures the capacity of *Xenopus* oocytes to mature over a 24-h period for cost-effectively evaluating the potential impact of complex environmental mixtures from confined animal feeding operations on the development, specifically sexual differentiation, of potentially exposed aquatic life.

•The primary objective of the proposed research program is to develop, a short-term, *in vitro Xenopus* oocyte maturation test kit for evaluating the chronic impact of xenobiotics in confined animal feeding operation waste on sexual development.

- •Three test compounds and three complex mixtures will be evaluated during the proposed project.
- •In tier 1 of a two-tiered approach, six test materials or environmental test samples from CAFOs will be used in the proposed project, culminating in the preparation of a standard guidance document for the performance of the proposed test method.
- •In tier 2, joint binary mixtures studies will be used to evaluate the joint responses of known EDCs and the complex wastewaters, as well as, potential for interaction.
- •A radioreceptor (OMPR and AR) binding assay will be used to determine if the effects induced are the result of alteration in steroid hormone receptor binding.

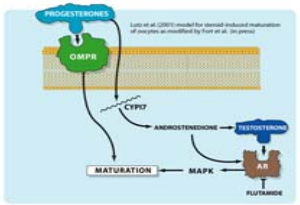
Expected Results

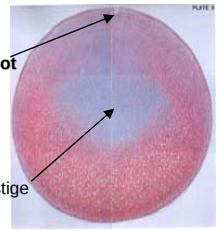
•We anticipate the development of a commercial test kit that can be used to screen CAFO run-off for endocrine disrupting capacity.

•We expect that the kit will also provide relevant information regarding hazards to aquatic biota downstream of CAFO run-off. This information will be valuable in establishing potential ecological risks to aquatic life.

•We anticipate that this technology could be used within a regulatory testing framework to establish guidelines and toxics-based water quality standards for CAFO run-off.

Oocyte Maturation Model



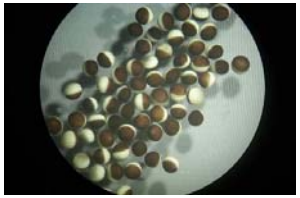


Potential Environmental Benefits

•By validating and commercializing *in vitro* oocyte maturation assay test kit for testing for pharmaceutical and hormone activities in waste run-off from animal feed operations, we will provide the scientific and regulatory communities with a versatile assay using an "environmentally relevant" species. Since no other rapid assay currently exists which is capable of rapidly and cost-effectively measuring EDCs (progestin and androgen) in the environment, and little is currently know regarding the potential impacts of animal feeding operation waste; waste samples will eventually require testing of this type. As opposed to many other advanced technologies, this bioassay can be developed, validated, and marketed quickly

Oocyte Collection

Banded Stage VI Oocytes



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